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EFFECTS OF GAMMA RADIATION EMITTED BY COBALT 60 ON OVULATION, FERTILIZATION, AND EMBRYONAL DEVELOPMENT OF THE FROG

[Comment: The following report was submitted for publication by Academician L. A. Orbeli on 6 November 1954 and was published as a contribution from Academician L. A. Orbeli's Group for Individual Work [or Special Studies] (Gruppa dlya Individual'noy Raboty Akademika L. A. Orbeli), Academy of Sciences USSR. No publications emanating from this group have been noted before. The special research unit headed by Academician Orbeli is either new or has not published anything up to now, as far as known. The work described below deals with the sterility effects produced by gamma radiation and by radioactive cobalt. The creation of a special academy unit engaged in research on the harmful effects of gamma radiation, and presumably also of other types of radiation, in producing sterility and exerting other injurious effects would be of interest because of the bearing which this type of research has on atomic energy work. It is not known at this stage whether the group in question does research on the physiological effects of radiation and of radioactive isotopes only or is engaged in other investigations as well.]

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EFFECTS OF GAMMA RADIATION EMITTED BY COBALT 60 ON OVULATION, FERTILIZATION, AND EMBRYONAL DEVELOPMENT OF THE FROG

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The methods of artificially inducing ovulation and of artificial insemination, as developed by Kashchenko in Nemilov's laboratory, were used to study the effects of gamma radiation emitted by cobalt 60. Female frogs which had ova in their ovaries in the winter but were not yet ready for fertilization, and males which already had mature spermatozoa were subjected to irradiation with gamma rays. The source of the radiation was Co^{60}. Over a period of 48 hours, the exposed frogs received a sublethal dose of between 6,800 and 7,500 r.

In the first series of experiments, on the second day after irradiation, a hypophysis suspension was injected into the abdominal cavities of the females to induce ovulation. In one case, a suspension of triturated hypophyses taken from two other irradiated females was injected into the abdominal cavity of an irradiated female. In a second case, an irradiated female was injected with a suspension of hypophyses taken from nonirradiated females. In a third case, a nonirradiated, healthy female was injected with a suspension of hypophyses taken from irradiated females. Finally, in the fourth case, which served as a control, a nonirradiated female was injected with a suspension of hypophyses taken from nonirradiated females.

After 48 hours, the hypophysis-suspension injections were repeated in the same combinations. Forty-eight hours after the second injections, the abdominal cavities of the irradiated females were dissected and the results of the described experiments were recorded.

The experiments showed that, under the conditions of our experiments, the process of ovulation in the irradiated frogs was not suppressed and that there were not even any quantitative changes. In all three combinations, in which irradiated female recipients or hypophyses taken from females which had been irradiated were used, the speed of the ovulation process and the quantity of ovulated ova were identical with the same factors in the control animals.

The same results were obtained in the second series of experiments in which the process of ovulation was induced 12 days after irradiation.

The egg cells which ovulated under artificial conditions were found suitable for fertilization experiments. For this purpose, it was sufficient to open the uterine portion of the oviduct, remove the egg cells from it, and immerse them in water containing spermatozoa (the latter were obtained by crushing testes in the water). The consummation of fertilization is easily determined by the speed with which the animal poles of the egg cells turn upwards, since this happens to a significant degree more rapidly in the case of fertilized ova than in that of unfertilized ova.

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Artificial fertilization was carried out in the following combinations:

Group I. (Control ova from a nonirradiated female, ovulation in which had been induced by the injection of a suspension of hypophyses taken from nonirradiated females, and spermatozoa from a nonirradiated male

Group II. The same type of ova as Group I, and spermatozoa from an irradiated male

Group III. Ova from an irradiated female, ovulation in which had been induced by the injection of a suspension of hypophyses from nonirradiated females, and spermatozoa from an irradiated male

Group IV. The same type of ova as Group III, and spermatozoa from a nonirradiated male

Group V. Ova from a nonirradiated female, ovulation in which had been induced by the injection of a suspension of hypophyses from irradiated females, and spermatozoa from a nonirradiated male

Group VI. The same type of ova as Group V, and spermatozoa from an irradiated male

Group VII. Ova from an irradiated female, ovulation in which had been induced by the injection of a suspension of hypophyses from irradiated females, and spermatozoa from an irradiated male

Group VIII. The same type of ova as Group VII, and spermatozoa from a nonirradiated male

Fertilization experiments 5 and 16 days after irradiation showed that, in all combinations, and for both periods, fertilization occurred in a completely normal manner. In all instances, there was almost 100 percent fertilization of the ova (see Table 1, appended). Consequently, as in the process of ovulation, the fertilization process was not disrupted by ionizing radiation of the indicated dosage. This situation changes subsequently, however.

As can be seen from Table 1, in the control Group I, the four-cell blastomere stage was reached on the average by 87 percent of the ova; the neurula stage by 81 percent; the first movement stage (lateral curving of the embryo into a ring shape) by 79 percent; and the free-swimming stage by 75 percent.

In Group IV, in which the female was irradiated before ovulation was induced but the process of ovulation was induced by the injection of hypophyses taken from normal females, and fertilization was carried out with normal spermatozoa, the rate of survival was significantly lower. In this group, only 65 percent of the embryos reached the four-cell blastomere stage; 55 percent, the neurula stage; 52 percent, the first movement stage; and 34 percent, the free-swimming stage.

In Group II, in which the female was normal, and ovulation was induced in her by normal hypophyses, but fertilization of the ova was carried out with spermatozoa taken from irradiated males, the free-swimming stage was not reached by any of the embryos. The four-cell blastomere stage was reached by 55 percent; the neurula stage, by 13 percent; and the first movement stage, by only 6 percent of the embryos.

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Still lower results were obtained in the cases where fertilization was carried out 16 days after the irradiation of the animals. In Group IV, the process of development stopped at the neurula stage, and, even then, only 15 percent of the ova reached this stage. In Group II, only 7 percent reached the neurula stage.

In Group V, in which ovulation was induced in normal females by hypophyses taken from irradiated females on the twelfth day after irradiation, and fertilization was carried out with normal spermatozoa, the process of development also stopped at the neurula stage, and only 19 percent of the ova reached this stage. Only 66 percent of the ova reached the four-cell blastomere stage, contrasted with 96 percent in the case of the control group. Less clear-cut, but nevertheless discernible, is the dependence of embryonal development on the kind of hypophysis which induces ovulation. The dependence [on the state of the hypophysis] is also seen in cases where ovulation is induced on the day following irradiation of the hypophysis.

The effect of the disruption of the function of the hypophysis on embryogenesis can also be observed in other groups. For example, this is evident from comparing Group II with Group VI and Group III with Group VII. It can thus be seen that embryogenesis is more strongly disrupted, other conditions being equal, when ovulation is induced by hypophyses taken from irradiated females.

More pronounced harm to the process of embryogenesis is encountered in the combination used in Group VII, where both spermatozoa and ova are derived from irradiated frogs and ovulation is induced by injection of the hypophyses of irradiated frogs.

The data presented show that serious disruption of embryogenesis occurs when either the males or the females have been irradiated. However, after equal doses of irradiation, the destructive consequences of the irradiation of the male are more clearly expressed and appear earlier than the consequences of the irradiation of the female.

Inasmuch as the spermatozoa are at a mature stage at the time of irradiation, it can be assumed that penetrating irradiation has a direct harmful effect on them. The ova, under the conditions of the experiments, were still not ready for fertilization at the time of irradiation, and their maturation occurred after irradiation. In this case, damage to the function of the maternal organism was [more] significant.

Without denying the direct harmful effect of penetrating irradiation on the ova, which is attested to by the fact that embryogenesis was disrupted in the combinations in which irradiated females took part while the hypophyses inducing ovulation in them were normal, we should acknowledge the substantial role of damage to the function of the hypophysis. This is evident from the fact that to disrupt the process of embryogenesis, it is sufficient that the hypophyses used to induce ovulation in any particular combination be taken from irradiated frogs. In this case, although the ova have not been subjected to the action of penetrating irradiation, they, nevertheless, seem to be defective because their ovulation has been induced by irradiated hypophyses.

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It can be concluded from this that, under normal conditions, the function of the hypophysis has an effect not only on the quantitative aspects of the process of ovulation, i.e., the speed of the process and the number of ovulated ova, but also on its qualitative aspects. The quantitative aspects, with the doses of irradiation used in these experiments, are not disturbed, but the qualitative aspects suffer severely. Therefore, it must be recognized that one of the mechanisms which disrupts embryonal development, and which occurs even outside of the maternal organism, is damage by penetrating irradiation of the function of the maternal hypophysis.

[Table 1 follows:]

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Table 1. Survival of Embryos (%)

Group	Fertilization 5 Days after Irradiation				Fertilization 16 Days after Irradiation			
	4-Cell Blastomere Stage	Neurula Stage	Lateral Movement Stage	Free-Swimming Stage	4-Cell Blastomere Stage	Neurula Stage	Lateral Movement Stage	Free Swimming Stage
I	87	81	79	75	96	90	89	89
II	56	14	6	0	86	7	0	--
III	58	7	4	0	48	0	--	--
IV	65	55	52	34	58	15	0	--
V	76	63	56	38	66	19	0	--
VI	61	6	2	0	45	1	0	--
VII	38	5	0	--	35	0	--	--
VIII	66	63	50	34	42	19	0	--

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